

## Properties of Soilborne Wheat Mosaic Virus Isolates in Nebraska

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### ABSTRACT

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Three properties of soilborne wheat mosaic virus (SBWMV) isolates collected in Nebraska were compared: virus-specific proteins in the infected tissue, peptide maps of coat protein after partial chemical cleavage, and RNA patterns of deletion mutants in virions purified from individual plants. Procedures for these determinations were simplified for study of

viral strain variation. Virus isolates from six randomly selected fields in Nebraska were similar by these three criteria. The reason for variation in yield loss from field to field does not lie in presence of viral strains identifiable by rod length, properties of viral proteins, or propensity to mutate by deletion under controlled growing conditions.

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Soilborne wheat mosaic virus (SBWMV) has a bipartite RNA genome separately encapsidated in rod-shaped virions, 20 nm in diameter, with a capsid protein molecular mass of 19,700 daltons (25). Component I virions are always the same length, 281 nm, but component II virions vary from 92 to 138 nm in length. Virus isolated from naturally infected field plants in early spring always has component II virions 138 nm long and is called the wild type (WT) (2,25). Plants inoculated with WT virus by the vector (*Polymyxa graminis* Led.), by leaf rubbing with purified virions, or by leaf rubbing with viral RNA had component II virions 138 nm long when first infected, but after several months component II virions of shorter length predominated (26). These shorter component II virions are thought to arise by deletion mutation because the predominant length varied from plant to plant. Component II virions as short as 92 nm along with component I

virions can cause infection (25,26). Plants infected with WT virus have a 90-kdalton protein that is absent in plants infected by mutants (Y. Shirako and M. K. Brakke, *unpublished*). The 90 kdalton protein is a putative inclusion body protein coded by WT-SBWMV-RNA II, but not by mutant RNA II, as shown by the results of in vitro translation experiments (*unpublished*).

Knowledge of the role of virus strains in determining crop losses is important for devising and recommending control strategies. Losses caused by SBWMV appear to vary from field to field and have been estimated at 0-50% (4,11,21,22). Originally, it was presumed that some of this diversity arose from strain variation (22). This hypothesis was supported by the fact that plants from some fields had virus rods of two characteristic lengths and plants from other fields, rods of three lengths (3,22). Apparently stable two-component strains with virions of different characteristic lengths were separable from a plant containing three viral components (3,25). However, the utility of characterizing virus strains by rod length appears doubtful following the discovery that the distribution of lengths of virus rods from a single plant changes from month to month as a result of deletion mutations (25,26).

The difference in severity of symptoms, yield losses and virion compositions of SBWMV-infected winter wheats from field to field provoked an interest in investigating methods other than rod length

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for detection of strain difference of SBWMV. Three criteria have been used in this study: the accumulation of virus-specific proteins in the infected tissue; peptide maps of viral coat protein after cyanogen bromide (CNBr), formic acid (HCOOH), or hydroxylamine (NH<sub>2</sub>OH) cleavage; and the frequency of deletion mutation and the RNA patterns of mutants.

### MATERIALS AND METHODS

**Virus sources.** Wheat plants infected with SBWMV-WT were either collected from the SBWMV nursery in Lincoln in March or were obtained by inoculating seedlings grown in the growth chamber with root debris from infested soil (6). Lab 1 and Lab 2 mutants with component II virions 92 and 110 nm long, respectively, were naturally occurring variants selected earlier (25,26) and were maintained on Michigan Amber wheat in the growth chamber at 18 C by mechanical transfer. Thirty SBWMV-infected wheat plants were collected from six randomly selected fields in Nebraska and transplanted individually in a growth chamber.

**Virus purification.** The purification procedure was derived from our earlier methods (3,26). Frozen infected leaves were ground in 0.5 M sodium borate, pH 9.0, 1 mM EDTA and filtered through cheesecloth. The supernatant after the low-speed centrifugation was clarified by adding K<sub>2</sub>HPO<sub>4</sub> and CaCl<sub>2</sub> to 20 mM with stirring and again centrifuging at low speed. After adding Triton X-100 to 2%, the supernatant was centrifuged through a 20% sucrose pad at 28,000 rpm for 2 hr in a Beckman type 30 rotor. The virus in the resuspended pellet was further purified by centrifugation in a 10–40% sucrose density gradient made in 0.5% Triton X-100.

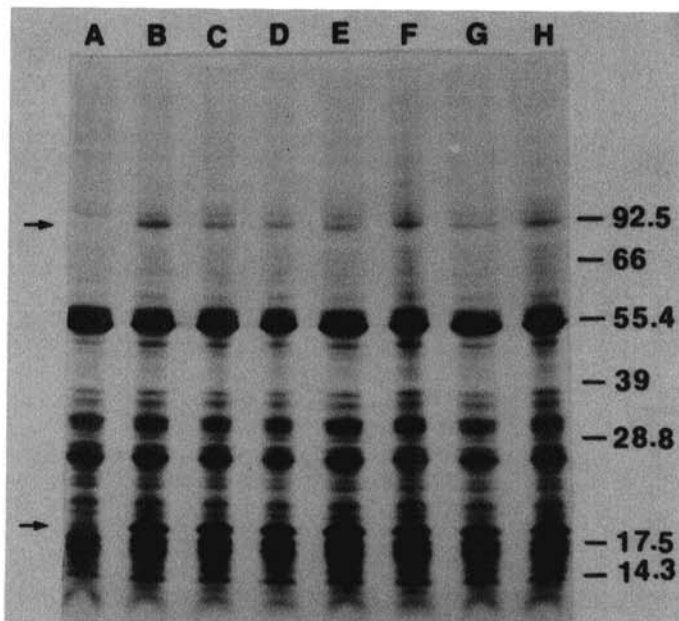
**Virus-specific proteins.** One gram of fresh, fully expanded, infected leaves was homogenized in 10 ml of 50 mM sodium orthoborate, pH 8.0. The homogenate was filtered through Miracloth and centrifuged directly at 32,000 rpm for 1 hr in a Beckman Ti 50 rotor without a preceding low-speed centrifugation. Nonpelleted proteins in the supernatant were precipitated by

adding methanol. Proteins in both fractions were analyzed by gel electrophoresis. Proteins from healthy leaves of the same age were prepared at the same time as a control.

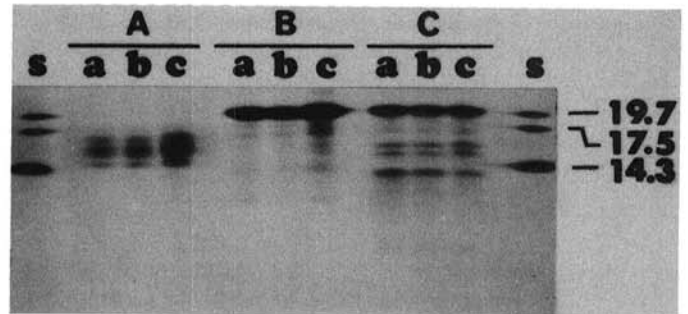
**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).** Protein samples were dissolved in cracking buffer (15) and heated at 100 C for 3 min. Electrophoresis was performed by using the Laemmli buffer system (12). The gels were stained with Coomassie Blue R or silver nitrate (19).

**Peptide mapping.** Procedures for partial chemical cleavage of purified virions or coat protein in polyacrylamide gel strips were adapted from those described (1,13,14,20). Hydroxylamine cleaves at asparaginyl-glycyl, formic acid at aspartyl-prolyl, and cyanogen bromide at methionine residues. Proteins of type-strain tobacco mosaic, and brome mosaic viruses with known amino acid sequence were used to optimize reaction conditions, primarily time and temperature of treatment. The virus concentration in each reaction was adjusted to 1 mg/ml. The CNBr cleavage was carried out at room temperature for 1 hr with 20 mg of reagent per milliliter in 70% HCOOH and terminated by neutralization with ammonium hydroxide and methanol precipitation. Proteins were cleaved with 70% HCOOH at 37 C for 24 hr and the cleavage was terminated as for the CNBr reaction. The NH<sub>2</sub>OH cleavage was carried out at 45 C for 8 hr with 2 M NH<sub>2</sub>OH in 0.2 M Na<sub>2</sub>CO<sub>3</sub>, pH 9.0, and terminated by methanol precipitation. For quick assay of many field samples, a two-step SDS-PAGE system was used. Virions were partially purified through one cycle of differential centrifugation. About 1–2 μg of coat proteins were first separated in a SDS-10% PAGE. After staining with Coomassie Blue R, the gel strip containing the coat proteins was cut out and rinsed with distilled water. Chemical cleavages were performed by placing the gel strip in a test tube filled with cleavage reagent under the same conditions used for cleaving the purified virions. Reagents rapidly diffuse into or out of the gel strip, but proteins and peptides diffuse out slowly. Under the conditions used, ample peptides remain for detection by sensitive silver staining. To terminate the CNBr and HCOOH reaction, the gel strip was neutralized with several changes of 1 M tris and visualized with bromophenol blue as indicator. To terminate the NH<sub>2</sub>OH cleavage, the gel strip was washed with distilled water. All gel strips were incubated in protein cracking buffer for 30 min at 25 C before analysis for the cleavage products in a second SDS 8–25% PAGE. The gel strip was placed directly over the stacking gel which was poured without sample wells. Identical peptide patterns for two proteins means they have target amino acids at identical positions and that rates of cleavage by the chemicals are the same.

**Viral RNA composition.** The RNA preparation and gel electrophoresis were as described (25). The virus samples from each individual plant were purified through one cycle of differential centrifugation and freezing. RNA was prepared from the virus



**Fig. 1.** SDS-PAGE of soilborne wheat mosaic virus (SBWMV)-specific proteins in infected wheat leaves. Proteins of high-speed centrifugation pellets were prepared as described in the text. Lane A, proteins extracted from healthy plants; lane B, proteins extracted from wild-type SBWMV (SBWMV-WT)-infected plants maintained in an SBWMV nursery; and lanes C–H, proteins extracted from SBWMV-infected plants from six different fields. The arrows indicate two virus-specific proteins. Numbers indicate the molecular masses ( $\times 10^{-3}$  daltons) of protein markers. Each lane has protein from 20 mg of leaves. Proteins in the gels were stained with Coomassie Blue.



**Fig. 2.** Peptide maps of coat proteins of the wild-type soilborne wheat mosaic virus (SBWMV-WT) and its Lab 1 and Lab 2 mutants. Group A, CNBr cleavage; group B, HCOOH cleavage; and group C, NH<sub>2</sub>OH cleavage. The cleavage products were analyzed on a SDS-8 to 25% polyacrylamide gel and stained with Coomassie Blue. In each group, a, b, and c are peptide maps of SBWMV-WT, Lab 2, and Lab 1, respectively. The markers under s are SBWMV-WT coat protein, TMV-type coat protein, and lysozyme, respectively; the numbers indicate their molecular masses ( $\times 10^{-3}$  daltons).

suspension by SDS disruption, phenol extraction and formaldehyde denaturation and analyzed on a 2.0% polyacrylamide-0.5% agarose composite gel.

## RESULTS

**Identification of virus-specific proteins.** Virus-specific proteins in the infected leaf extract were detected by SDS-PAGE. Analysis of a high-speed centrifugation pellet, without preceding clarification by low-speed centrifugation, allowed simultaneous determination of the 19.7 kdalton coat protein and another 90 kdalton protein in wheat infected with SBWMV-WT (Fig. 1, Lane B). These two proteins were absent from healthy wheat tissue (Lane A). Samples from six different fields gave the same protein profile on SDS-PAGE as that obtained for wheat infected with type SBWMV-WT (Lanes C-H), indicating those samples were infected by SBWMV-WT. There was no distinguishable virus-specific protein in the methanol-precipitated fraction (*unpublished*).

**Peptide mapping of coat protein.** Peptides generated by chemical cleavage of coat proteins of various SBWMV isolates were analyzed on an SDS-8 to 25% PAGE. All three chemical cleavage

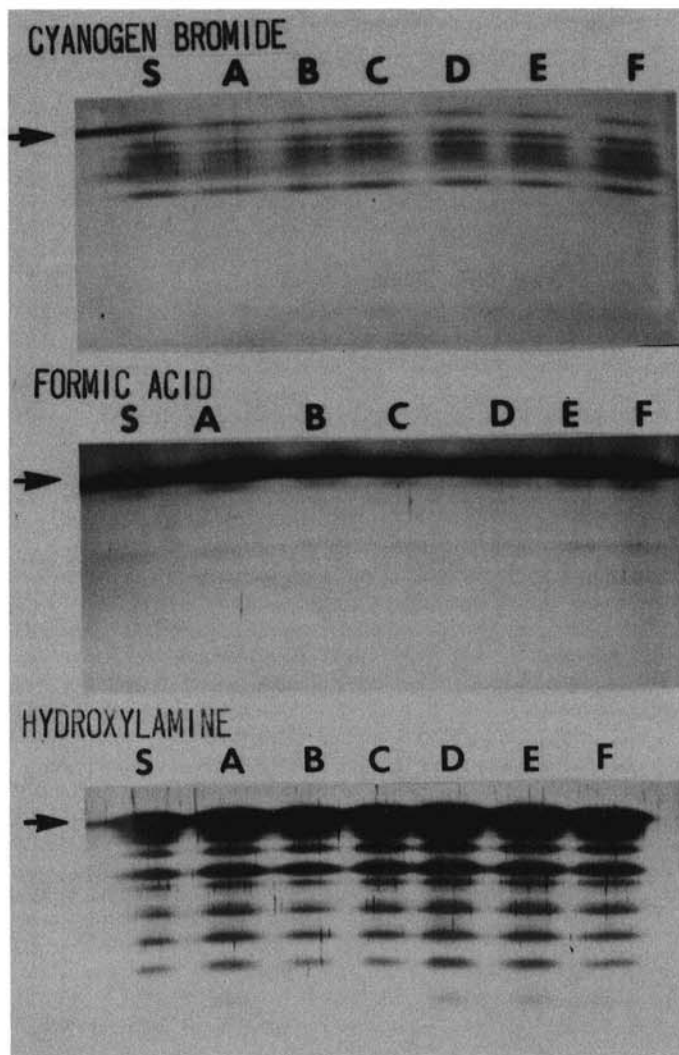
patterns of Lab 1 and Lab 2 mutants were the same as that of wild type (Fig. 2), indicating no deletions within the coat protein. Chemical cleavage of the coat proteins in SDS-PAGE strip not only simplified the cleavage procedure but allowed analysis of at least 24 samples in a regular 12×15-cm gel under uniform reaction conditions. All three chemical cleavage patterns of the six field samples were the same as for wild type (Fig. 3). The similarity of the peptide maps of all isolates indicates they have the same coat protein. It may be noted that reproducibility of the peptides obtained by partial chemical cleavage is excellent for proteins present in a single strip, but varies slightly from experiment to experiment (compare Fig. 2 and Fig. 3).

**Viral RNA compositions.** Individually transplanted SBWMV-infected plants from six different fields were maintained in a growth chamber for 4 mo. Viral RNAs from individual plants were analyzed at the time of collection, 1 mo later, and 4 mo later. Amounts of RNA loaded on a gel were about one-tenth of that obtained from virus purified from 1-2 g of infected leaves. Variations in concentration of RNA among the samples might come from variation in virus concentration or recovery during purification. Only the wild-type RNAs were detected in samples from all plants at the time of collection and after 1 mo in the growth chamber (*unpublished*). But after 4 mo in the growth chamber, RNA compositions varied from plant to plant. The pattern of mutation was similar in plants from each field examined (Fig. 4).

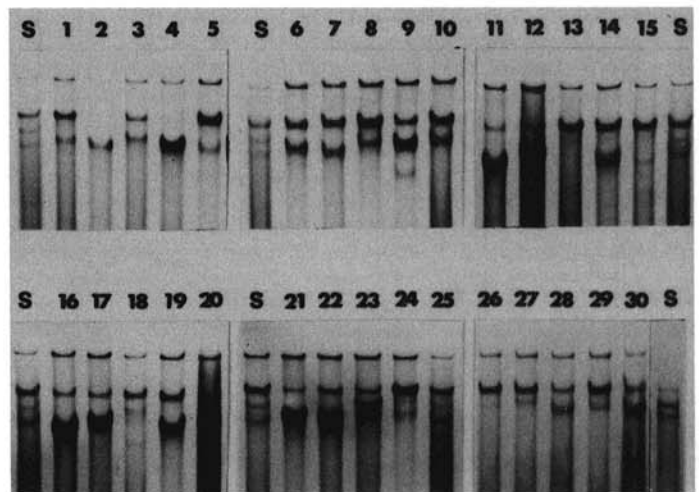
## DISCUSSION

Growing resistant wheat cultivars is the only practical method to control SBWMV. Understanding the properties of SBWMV strains is important in devising strategies for breeding resistant varieties. In earlier reports related to this disease, multiple SBWMV strains have been assumed, but only few attempts have been made to characterize strain variation (9,18,25,27). In this study we determined three properties of SBWMV in Nebraska and devised simplified procedures for determining these properties.

Two virus-specific proteins prepared by one-step centrifugation can serve as markers to characterize infection by SBWMV-WT. The 90-kdalton protein presumably is the inclusion body in the infected tissue (9,23), but proof is lacking. The 90-kdalton protein is absent in wheat infected with the Lab 1 and Lab 2 mutant infected wheat (Y. Shirako and M. K. Brakke, *unpublished*). Therefore, this protein is specific for WT virus. Serological relationships among



**Fig. 3.** Peptide maps of coat proteins of soilborne wheat mosaic virus (SBWMV) from different fields. Coat proteins first fractionated on an SDS-10% polyacrylamide gel were subsequently partially cleaved chemically in a gel strip. The cleavage products were analyzed on an SDS-8 to 25% polyacrylamide gel and stained with silver nitrate. The top gel shows CNBr cleavage; the middle gel, HCOOH cleavage, and the bottom gel, NH<sub>2</sub>OH cleavage. Lane S, wild-type soilborne wheat mosaic virus (SBWMV-WT) from the nursery, and lanes A to F, SBWMV from six different fields. The arrow marks the location of uncleaved protein.



**Fig. 4.** RNA compositions of soilborne wheat mosaic virus (SBWMV) from individual plants. SBWMV-infected plants from six different fields were transplanted and maintained in a growth chamber at 18 C for 4 mo. RNAs extracted from purified virions of these plants were denatured by formaldehyde and analyzed on polyacrylamide-agarose composite gels. Lane S, RNA mixture prepared from wild-type SBWMV (SBWMV-WT) and its Lab 1 and Lab 2 mutants. Lanes 1-30, RNAs from individual plants; every five consecutive numbers are a set of samples from one field.

inclusion bodies have so far been studied only for potyviruses (24). Comparisons of the 90-kdalton protein of SBWMV with those of similar viruses should be useful in classification.

Restriction endonuclease cleavage patterns readily identify and distinguish DNA molecules. By a similar principle, establishing the peptide maps of the coat protein should be a useful tool for characterizing a virus. In addition to characterizing the peptides by size by SDS-PAGE, one might also characterize them by isoelectric focusing to detect changes in amino acids with ionized side chains. Grouping of virus isolates by limited enzymatic proteolysis has been reported for tymoviruses and cucumoviruses and shown to be more useful than serology (5,10). We prefer partial chemical proteolysis for several reasons. First, the chemicals cleave the protein at defined amino acid residues or linkages by known mechanisms (1,7,14). Second, the chemical reaction is less conformation-dependent than enzymatic reactions, i.e., more reproducible. Third, there are a number of site-specific chemical reagents available for peptide mapping (8,16,17). Treating the coat proteins in a gel slice simplifies handling several samples at one time, assures uniform reaction conditions, and requires minute amounts. An amount of protein that was optimal for staining by Coomassie Blue gave amounts of peptides readily stained by silver nitrate.

Results of the RNA analysis reveal that field samples collected in the early spring contain only SBWMV-WT. We suspect the frequency of deletion mutation in plants in different fields may vary. We found that virus from each of the six fields produced deletion mutants in growth chambers. It may be that under natural conditions virus from some fields mutates faster than that from others. Since the Lab 1 and Lab 2 mutants cause more severe symptoms on wheat than does SBWMV-WT (25), differential accumulation of mutants may lead to the different degree of symptom severity and yield losses. The ecology of SBWMV in the field is still unclear.

There was no detectable variation of viruses in this study showing that these fields were infected with similar strains of SBWMV. Further application of these methods to viruses from different fields with different degrees of yield loss may show whether that loss is due to strains of the virus or to some other causes.

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